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The amendment made in the specification corrects the inadvertent error of the continuing data and addition of sequence identifiers in the subject application as requested by the Examiner.

The amendments made to claims 53 and 70 are merely to clarify the invention and do not introduce new matter. New claims 71-85 are merely recite individual members of the Markush group recited in claim 70, and therefor add no new matter.

Support for claim 53 can be found, for example, on page 22, lines 26-30, continuing to page 23, lines 1-22.

Support for claim 70 can be found, for example, on page 47, lines 12-17; page 14, lines 26-28; page 22, line 26 through page 23, line 22; page 44, lines 17-19; and, page 127, lines 8-11.

Concerning claim 70, add claims 71-85 dependent therefrom, Applicants note that the application as-filed discloses fusion proteins that comprise PSCA or fragments thereof fused to heterologous amino acids. This subject matter is disclosed, for example, at page 46-47. In the paragraph spanning page 46-47, it is disclosed that PSCA or a fragment of PSCA is a peptide agent as used in the application. In the paragraph at page 47, lines 12-17, it is disclosed that PSCA or fragments thereof (peptide agents) can be fused to heterologous amino acids. An example of a PSCA fragment fused to heterologous amino acids is depicted graphically in Figure 50. Furthermore, examples of heterologous amino acids that provide for detectable markers such as epitope tags are disclosed in the application as-filed, e.g., at page 14, lines 27-28; page 41, lines 8-11; page 44, lines 17-19; page 127, lines 8-11; and, page 128, lines 27-31, as well as Figure 50. The use of an agent such as fusion protein comprising PSCA or a fragment thereof in cell-free or cellular assay systems is disclosed, e.g., at page 46, lines 5-8; for example, cellular systems are used to active or characterize immune system cellular responses. Moreover,

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Example 20 at pages 126-127 discloses a system used to characterize PSCA levels, including use of a fusion protein comprising PSCA and heterologous amino acids as a standardization reagent.

Accordingly, these changes do not involve new matter and applicants respectfully request entry of these changes.

No fee is due in connection with this Supplemental Amendment. However, if a fee is deemed necessary, applicants authorize the Patent Office to charge the fee to the Deposit Account No. 50-0306.

Respectfully submitted,



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**VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE  
 SPECIFICATION**

**In the Specification:**

On page 1, please replace the first paragraph, starting at line 5 and beginning "This application is" with the following:

-- This application is a Divisional application of U.S. Serial No. 09/564,329 filed May 3, 2000, which is a continuation-in-part (CIP) of U.S. Serial No. 09/359,326 filed July 20, 1999, now abandoned, which is a CIP of U.S. Serial No. 09/318,503 filed May 25, 1999, now issued U.S. Patent No. 6,261,791, issued on July 17, 2001, [based on U.S. Serial No. 09/318,503, filed May 25, 1999,] which is a CIP of U.S. Serial No. 09/251,835 filed February 17, 1999, now issued U.S. Patent No. 6,261,789, issued on July 17, 2001, [based on U.S. Serial No. 09/251,835, filed February 17, 1999,] which is a CIP of U.S. Serial No. 09/203,939 filed December 2, 1998, now issued U.S. Patent No. 6,258,939, issued on July 10, 2001, [based on U.S. Serial No. 09/203,939, filed December 2, 1998,] which is a CIP of U.S. Serial No. 09/038,261 filed March 10, 1998, now issued U.S. Patent No. 6,267,960, issued July 31, 2001, [based on U.S. Serial No. 09/038,261, filed March 10, 1998,] claiming the priority of provisional applications, U.S. Serial No. 60/228,816 filed March 10, 1997; U.S. Serial No. 60/071,141 filed January 12, 1998 and[;] U.S. Serial No. 60/074,675 filed February 13, 1998. Further, t[T]his application [further] claims the priority of U.S. Serial No. 09/564,329 filed May 3, 2000, which claims the benefit of the filing dates of U.S. Serial No. 60/113,230 filed December 21, 1998 [U.S. Serial No. 60/124,658 filed March 16, 1999], U.S. Serial No. 60/120,536 filed February 17, 1999 and U.S. Serial No. 60/124,658 filed March 16, 1999 [U.S. Serial No. 60/113,230 filed December 21, 1998]. The contents of all [of] the foregoing applications are incorporated by reference into the present application. --

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Please replace the paragraph at page 30, lines 17-26 with the following rewritten paragraph:

-- The amino acid sequence of PSCA presented herein may be used to select specific regions of the PSCA protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of the PSCA amino acid sequence may be used to identify hydrophilic regions in the PSCA structure. Regions of the PSCA protein that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. Fragments containing these residues are particularly suited in generating specific classes of anti-PSCA antibodies. Particularly useful fragments include, but are not limited to, the sequences TARIRAVGLLTVISK (SEQ. ID NO.: 16) and SLNCVDDSDQDYVVGK (SEQ. ID NO.: 18). --

Please replace the paragraph at page 38, lines 2-5 with the following rewritten paragraph:

-- Fragments of human *PSCA* that are particularly useful as selective hybridization probes or PCR primers can be readily identified from the entire *PSCA* sequence using art-known methods. One set of PCR primers that are useful for RT-PCR analysis comprise 5' - TGCTTGCCCTGTTGATGGCAG - (SEQ. ID NO.: 19) and 3' - CCAGAGCAGCAGGCCGAGTGCA - (SEQ. ID NO.: 20). --

Please replace the paragraph at page 66, lines 16-22 with the following rewritten paragraph:

-- In preferred embodiments, DNA fragments of 9kb, 6kb, 3kb, and 1kb derived from the 5' upstream region of the PSCA gene, as shown in Figure 42, were produced by techniques described herein. The 9kb PSCA upstream region (pEGFP—PSCA) is involved with gene regulatory activity and was deposited on May 17, 1999 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and has there been identified as follows ATCC No. PTA-80. The 9 kb fragment was obtained by

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amplification using a T7 primer and RHPSCA3'-5 (5'-gggaattcgacagccttcagggtc-3')  
 (SEQ ID NO. 21). --

Please replace the paragraph at page 80, lines 15-29 with the following rewritten paragraph:

-- RDA, Northern Analysis and RT-PCR: Representational difference analysis of androgen dependent and independent LAPC-4 tumors was performed as previously described (Braun et al., 1995, Mol. Cell. Biol. 15: 4623-4630). Total RNA was isolated using Ultraspec<sup>R</sup> RNA isolation systems (Biotecx, Houston, TX) according to the manufacturer's instructions. Northern filters were probed with a 660bp RDA fragment corresponding to the coding sequence and part of the 3' untranslated sequence of PSCA or a ~400bp fragment of PSA. The human multiple tissue blot was obtained from Clontech and probed as specified. For reverse transcriptase (RT)-PCR analysis, first strand cDNA was synthesized from total RNA using the GeneAmp RNA PCR core kit (Perkin Elmer-Roche, New Jersey). For RT-PCR of human PSCA transcripts, primers 5'-tgcttgccctgttgatggcag- and 3'-ccagagcagcaggccgagtgca- were used to amplify a ~320 bp fragment. Thermal cycling was performed by 25-25 cycles of 95° for 30 sec, 60° for 30sec and 72° for 1 min, followed by extension at 72° for 10 min. Primers for GAPDH (Clontech) were used as controls. For mouse PSCA, the primers used were 5' -ttctcctgctggccacctac- and 3' -gcagctcatcccttcacaat- (SEQ ID NO. 19 and SEQ ID NO. 20 respectively). --

Please replace the paragraph at page 89, lines 7-17 with the following rewritten paragraph:

-- Generation and Production of Monoclonal Antibodies: BALB/c mice were immunized three times with a purified PSCA-glutathione S-transferase (GST) fusion protein containing PSCA amino acids 22-99 (FIG. 1B). Briefly, the PSCA coding sequence corresponding to amino acids 18 through 98 of the human PSCA amino acid sequence was PCR-amplified using the primer pair:

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5'- GGAGAATTCATGGCACTGCCCTGCTGTGCTAC (SEQ ID NO. 22)  
 3'-GGAGAATTCCTAATGGGCCCCGCTGGCGTT (SEQ ID NO. 23)

The amplified PSCA sequence was cloned into pGEX-2T (Pharmacia), used to transform *E. coli*, and the fusion protein isolated. --

Please replace the paragraph at page 107, lines 2-26 with the following rewritten paragraph:

-- The reporter gene vectors are depicted in Figure 42 and were constructed as follows. The 14 kb Not I fragment was sub-cloned from the  $\lambda$  vector into a Bluescript KS vector (Stratagene), resulting in the pBSKS-PSCA (14kb) construct. The PSCA upstream sequence was subcloned from pBSKS-PSCA (14 kb) by PCR amplification using a primer corresponding to the T7 sequence contained within the Bluescript vector, and a primer corresponding to a sequence contained within PSCA exon 1 (primer H3hPSCA3'-5, the sequence of this primer is as follows: The sequence of H3hPSCA3'-5 is 5'-gggaagcttgacacagccttcagggtc-3' (SEQ ID NO. 24). The primer corresponding to PSCA exon 1 contained an introduced HindIII sequence to allow further subcloning following PCR amplification. The resulting amplified fragment was digested with HindIII and was subcloned into the pGL3-basic vector (Promega) to generate pGL3-PSCA (7 kb) which was used to generate a series of deletion reporter gene constructs containing varying lengths of PSCA upstream sequences operatively linked to the luciferase gene (Figure 42). The deleted portions of the PSCA upstream regions were obtained by subcloning restriction fragments from pGL3-PSCA (7 kb). The PSCA upstream region between -9 kb and -7 kb was subcloned from the pBSKS-PSCA (14 kb) construct, the Not I site was converted into a blunt end by Klenow and the fragment was cloned into the SacI/HindIII sites of pGL-PSCA (7 kb) in order to obtain the pGL3-PSCA (9 kb) construct. The reference to the sequences upstream of the PSCA coding region, such as -9 kb and -6 kb (etc.), are relative to the ATG start translation codon. The reporter gene constructs pGL3-

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PSCA (9 kb), pGL3-PSCA (6 kb), pGL3-PSCA (3 kb), and pGL3-PSCA (1 kb) were operatively linked to the luciferase gene (Figure 42). Plasmid, pGL3-CMV contains the cytomegalovirus promoter (Boshart, M. et al., 1985 *Cell* 41:521-530) linked to the luciferase gene and was used as a positive control. Also, plasmid pGL3 contains no promoter sequence and was used as a negative control plasmid. --

Please replace the paragraph at page 127, lines 20-31, and continuing to page 128, line 2 with the following rewritten paragraph:

-- The nucleotide sequences of the genes encoding the heavy chain variable regions of murine monoclonal antibodies 1G8, 4A10 and 2H9 were determined using the methods described in Coloma et al., 1992, *J Immunol. Methods* 153: 89-104. Primers for heavy chain variable region sequencing of mAbs 1G8 and 4A10 were as follows:

HLEAD.1: ggc gat atc cac cat ggR atg Sag ctg Kgt Mat Sct ett (SEQ ID NO. 25)

CH3': agg gaa ttc aYc tcc aca cac agg RRc cag tgg ata gac (SEQ ID NO. 26)

Primers for heavy chain variable region sequencing of mAb 2H9 were as follows:

HLEAD.2: ggg gat atc cac cat gRa ctt cgg gYt gag ctK ggt ttt (SEQ ID NO.27)

CH3': agg gaa ttc aYc tcc aca cac agg RRc cag tgg ata gac (SEQ ID NO. 26), --

#### **In the claims:**

For convenience, all pending claims are presented, whether amended or not, in the attached marked set of claims. Please amend claims 53 and 70, and add new claims 71-85 as follows:

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--53. (3X amended) A protein fragment consisting of the amino acid residues selected from the group consisting of:

- a. amino acid residues 2 through 50 as described in SEQ ID NO:2;
- b. amino acid residues 85 through 123 as described in SEQ ID NO:2;
- c. amino acid residues 46 through 109 as described in SEQ ID NO:2;
- d. amino acid residues 18 through 98 as described in SEQ ID NO:2;
- e. amino acid residues 22 through 99 as described in SEQ ID NO:2;
- f. amino acid residues 21 through 50 as described in SEQ ID NO:2;
- g. amino acid residues 46 through 85 as described in SEQ ID NO:2;
- h. amino acid residues 50 through 64 as described in SEQ ID NO:2;
- i. amino acid residues 67 through 81 as described in SEQ ID NO:2;
- j. amino acid residues 21 through 99 as described in SEQ ID NO:2;
- k. amino acid residues 71 through 82 as described in SEQ ID NO:2;
- l. amino acid residues 85 through 99 as described in SEQ ID NO:2;
- m. amino acid residues 18 through 50 as described in SEQ ID NO:2;
- n. amino acid residues 46 through 98 as described in SEQ ID NO:2; or
- o. amino acid residues 85 through 98 as described in SEQ ID NO:2. --

--55. (not amended) The protein fragment of claim 53, consisting of amino acid residues 2 through 50 as described in SEQ ID NO:2.--

--56. (not amended) The protein fragment of claim 53, consisting of amino acid residues 85 through 123 as described in SEQ ID NO:2.--

--57. (not amended) The protein fragment of claim 53, consisting of amino acid residues 46 through 109 as described in SEQ ID NO:2.--

--58. (not amended) The protein fragment of claim 53, consisting of amino acid residues 18 through 98 as described in SEQ ID NO:2.--



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- 59. (not amended) The protein fragment of claim 53, consisting of amino acid residues 22 through 99 as described in SEQ ID NO:2.--
- 60. (not amended) The protein fragment of claim 53, consisting of amino acid residues 21 through 50 as described in SEQ ID NO:2.--
- 61. (not amended) The protein fragment of claim 53, consisting of amino acid residues 46 through 85 as described in SEQ ID NO:2.--
- 62. (not amended) The protein fragment of claim 53, consisting of amino acid residues 50 through 64 as described in SEQ ID NO:2.--
- 63. (not amended) The protein fragment of claim 53, consisting of amino acid residues 67 through 81 as described in SEQ ID NO:2.--
- 64. (not amended) The protein fragment of claim 53, consisting of amino acid residues 21 through 99 as described in SEQ ID NO:2.--
- 65. (not amended) The protein fragment of claim 53, consisting of amino acid residues 71 through 82 as described in SEQ ID NO:2.--
- 66. (not amended) The protein fragment of claim 53, consisting of amino acid residues 85 through 99 as described in SEQ ID NO:2.--
- 67. (not amended) The protein fragment of claim 53, consisting of amino acid residues 18 through 50 as described in SEQ ID NO:2.--

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- 68. (not amended) The protein fragment of claim 53, consisting of amino acid residues 46 through 98 as described in SEQ ID NO:2.--
- 69. (not amended) The protein fragment of claim 53, consisting of amino acid residues 85 through 98 as described in SEQ ID NO:2.--
- 70. (Amended) A [recombinant] fusion protein [comprising] consisting of a Prostate Stem Cell Antigen (PSCA) protein fragment fused to heterologous amino acids, wherein the PSCA protein fragment consists of [a protein] the amino acid residues selected from the group consisting of:
  - a. amino acid residues 2 through 50 as described in SEQ ID NO:2;
  - b. amino acid residues 85 through 123 as described in SEQ ID NO:2;
  - c. amino acid residues 46 through 109 as described in SEQ ID NO:2;
  - d. amino acid residues 18 through 98 as described in SEQ ID NO:2,;
  - e. amino acid residues 22 through 99 as described in SEQ ID NO:2;
  - f. amino acid residues 21 through 50 as described in SEQ ID NO:2;
  - g. amino acid residues 46 through 85 as described in SEQ ID NO:2;
  - h. amino acid residues 50 through 64 as described in SEQ ID NO:2;
  - i. amino acid residues 67 through 81 as described in SEQ ID NO:2;
  - j. amino acid residues 21 through 99 as described in SEQ ID NO:2;
  - k. amino acid residues 71 through 82 as described in SEQ ID NO:2;
  - l. amino acid residues 85 through 99 as described in SEQ ID NO:2;
  - m. amino acid residues 18 through 50 as described in SEQ ID NO:2;
  - n. amino acid residues 46 through 98 as described in SEQ ID NO:2; or
  - o. amino acid residues 85 through 98 as described in SEQ ID NO:2. --
- 71. (New) The protein fragment of claim 70, consisting of amino acid residues 2 through 50 as described in SEQ ID NO:2. --

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- 72. (New) The protein fragment of claim 70, consisting of amino acid residues 85 through 123 as described in SEQ ID NO:2. --
- 73. (New) The protein fragment of claim 70, consisting of amino acid residues 46 through 109 as described in SEQ ID NO:2. --
- 74. (New) The protein fragment of claim 70, consisting of amino acid residues 18 through 98 as described in SEQ ID NO:2. --
- 75. (New) The protein fragment of claim 70, consisting of amino acid residues 22 through 99 as described in SEQ ID NO:2. --
- 76. (New) The protein fragment of claim 70, consisting of amino acid residues 21 through 50 as described in SEQ ID NO:2. --
- 77. (New) The protein fragment of claim 70, consisting of amino acid residues 46 through 85 as described in SEQ ID NO:2. --
- 78. (New) The protein fragment of claim 70, consisting of amino acid residues 50 through 64 as described in SEQ ID NO:2. --
- 79. (New) The protein fragment of claim 70, consisting of amino acid residues 67 through 81 as described in SEQ ID NO:2. --
- 80. (New) The protein fragment of claim 70, consisting of amino acid residues 21 through 99 as described in SEQ ID NO:2. --
- 81. (New) The protein fragment of claim 70, consisting of amino acid residues 71 through 82 as described in SEQ ID NO:2. --

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- 82. (New) The protein fragment of claim 70, consisting of amino acid residues 85 through 99 as described in SEQ ID NO:2. --
- 83. (New) The protein fragment of claim 70, consisting of amino acid residues 18 through 50 as described in SEQ ID NO:2. --
- 84. (New) The protein fragment of claim 70, consisting of amino acid residues 46 through 98 as described in SEQ ID NO:2. --
- 85. (New) The protein fragment of claim 70, consisting of amino acid residues 85 through 98 as described in SEQ ID NO:2. --